Medical and Pediatric Oncology 26:85–89 (1996)

Hepatotoxicity of 6-Mercaptopurine in Childhood Acute Lymphocytic Leukemia: Pharmacokinetic Characteristics

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Treatment with 6-mercaptopurine (6MP) is associated with adverse gastrointestinal (GI) and hepatic effects. Four patients, ages 6.9 ± 2.6 (mean ± S.D.) years, with acute lymphocytic leukemia (ALL) on maintenance chemotherapy including 6MP, developed nausea, vomiting, abdominal pain, elevated liver enzymes, and hyperbilirubinemia after 1.4 ± 1.0 (range 0.5–2) years. Liver biopsy in 1 patient was suggestive of drug-induced intrahepatic cholestatis. Symptoms resolved and liver function returned to normal after discontinuation of 6MP. Pharmacokinetic data of the symptomatic patients were compared with those of 25 ALL patients on the same protocol but without GI symptoms or hepatotoxicity. Levels of 6-thioguanine nucleotides (6-TGN) and the methylated metabolites of 6MP in red blood cells of the patients with hepatotoxicity, were not significantly different when compared to patients without hepatotoxicity, suggesting similar absorption of 6MP in both groups. Time to achieve peak 6MP levels was significantly longer in the symptomatic patients compared to the asymptomatic patients ($P = 0.005$). Peak levels and standardized concentration versus time curve (AUC) per 1 mg of 6MP per m^2 of body surface area were significantly lower in the patients with hepatotoxicity ($P = 0.016$; $P = 0.037$, respectively). A significant correlation between peak 6MP levels and standardized AUC ($r = 0.729$, $P < 0.0001$) was found. These results suggest accumulation of 6MP and its metabolites in the liver of the patients with GI symptoms, leading to hepatotoxicity.

Key words: acute lymphocytic leukemia, 6-mercaptopurine, hepatotoxicity

INTRODUCTION

6-mercaptopurine (6MP) is widely used for the treatment of childhood leukemia; however, its use is sometimes associated with adverse gastrointestinal and hepatic effects that may compromise the management of these patients [1–3]. Gastrointestinal side effects of 6MP include nausea, vomiting, diarrhea, anorexia, abdominal pain, and ulceration of the intestinal epithelium. Jaundice has been recorded in 6% to 42% of leukemic patients treated with this medication [1,4]. The mechanism of 6MP hepatotoxicity and jaundice remains obscure.

6MP is metabolized by S-methylation, which is catalyzed by thiopurine methyltransferase (TPMT), or by a multienzymatic process to form cytotoxic 6-thioguanine nucleotides (6-TGN) [5]. The bioavailability of oral 6MP is poor with great variability in the peak plasma concentration, in the time to peak concentration, and in the area under the concentration versus time curve (AUC) achieved after oral administration of a standard dose of the drug [6,7]. It has been documented that the poor bioavailability of oral 6MP is due to extensive first-pass metabolism by xanthine oxidase in the liver [6].

We report on 4 patients with acute lymphocytic leukemia (ALL) in remission, receiving maintenance therapy including 6MP, who developed severe adverse gastrointestinal and hepatic reactions. These patients had a significantly lower mean peak plasma concentration and lower AUC of 6MP compared to 25 other ALL patients in remission receiving the same treatment but who did not experience these adverse effects. We postulate that adverse hepatic effects in patients receiving 6MP may be due to an accumulation of 6MP and its metabolites in the liver.

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Received June 16, 1994; accepted November 14, 1994.

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PATIENTS AND METHODS

The pharmacokinetics of 6MP was studied in 29 patients with ALL in remission, on similar maintenance therapy with 6MP, methotrexate, vincristine, and prednisone. Four patients, aged 6.9 ± 2.6 (5.5–11; mean ± S.D., range) years had gastrointestinal symptoms and evidence of hepatic damage 1.4 ± 1.0 (range 0.5–2) years into therapy. Five other patients, ages 10.8 ± 5.0 (3–17) years, without gastrointestinal symptons or hepatitis, were investigated concomitantly to allow, in addition to pharmacokinetics of 6MP, the comparison of the patients with gastrointestinal damage in terms of TMPT and TGN activities. These five patients were chosen randomly among children treated at the same time for ALL with a similar protocol. An initial group of 20 patients without gastrointestinal symptoms who were studied and reported previously [7], were also included in the pharmacokinetic analysis. This group was treated with an identical protocol to the other nine children.

Using a previously described high performance liquid chromatography (HPLC) technique [8] for the measurement of 6MP concentration in plasma, we studied the pharmacokinetics of this drug. All patients fasted for 12 hours before receiving their dose of 6MP and took no medications for at least 24 hours prior to the study day. A baseline blood sample was obtained from each patient before the study dose of 6MP was administered. After oral dosing, 1 ml blood samples were drawn at 30 minutes and at 1, 1.5, 2.5, 3, 4, and 5 hours through a heparin lock. Plasma was separated immediately, frozen to −20°C, and analyzed on the same day. 6-TGN concentrations were measured by an HPLC assay described elsewhere [9] and TPMT activity was measured using a previously described method [10].

$AUC_{0-\infty}$ was calculated using the trapezoidal method. As the children were receiving different doses of 6MP, the dose of 6MP was normalized to dose of 1 mg/m^2. Correlation between various parameters was calculated by least-squares regression analysis. Data of the patients with gastrointestinal symptoms were compared to that of the patients without symptoms using the two-tailed Student t-test for unpaired results.

RESULTS

Four children with ALL in remission, ages 6.9 ± 2.6 (5.5–11) years, on maintenance therapy of 6MP, methotrexate, vincristine, and prednisone, developed nausea, vomiting, and abdominal pain 1.4 ± 1.0 (0.5–2) years into therapy. All patients had symptoms and signs of hepatic damage including jaundice, a tender enlarged liver, and elevated liver enzymes (AST, ALT, and GGT of 200 to 300 IU/L) and elevated total and direct bilirubin. One patient had a clinical picture consistent with hepatic encephalopathy with jaundice, unconsciousness, hypoglycemia, and hyperammonemia (88 μmol/L with normal up to 60 μmol/L). Laboratory results were as follows: AST 385 IU/L, ALT 389 IU/L, total bilirubin 243 μmol/L, direct bilirubin 213 μmol/L, PT 31.2 seconds (normal 10.5–13.5 seconds), and APTT 62.1 seconds (normal 24–40 seconds). A liver biopsy obtained from this patient was highly suggestive of drug-induced intraphepatic cholestasis. This diagnosis was supported by electron microscopy studies which demonstrated widespread changes in the mitochondria and near absence of glycogen. An attempt was made to exclude other possible causes of hepatitis in these patients. Titers of antibodies to hepatitis A, B, C, EBV, and CMV were negative. Symptoms resolved and liver function tests gradually returned to normal after discontinuation of 6MP. In all cases, rechallenge with 6MP resulted in the recurrence of symptoms and abnormal laboratory findings.

Five other patients with ALL in remission, aged 10.8 ± 5.0 years, receiving the same maintenance therapy as the first group, but without gastrointestinal symptoms, were evaluated. Their characteristics are sum -marized in Table I. Combining their data with that of the 20 ALL patients, previously studied and reported [7] none of whom had gastrointestinal toxicity, (Table II), showed that these 25 patients had significantly higher ($P = 0.016$) 6MP peak levels than the patients with gastrointestinal symptoms. The time to achieve peak 6MP levels was 70 ± 48 minutes in the asymptomatic patients as compared to a prolonged time of 135 ± 17 minutes in the symptomtic patients ($P = 0.005$). There was also a significant difference between the two groups in standardized AUC per 1 mg of 6MP per m^2 of body surface area: 370 ± 214.3 ngxmin/ml in the asymptomatic group as compared to 137.9 ± 56.5 ngxmin/ml in the symptomatic group ($P = 0.037$; Fig. 1). There was a significant correlation between peak 6MP levels and AUC per 1 mg of 6MP per m^2($r = 0.729$, $P < 0.0001$). There was no significant difference between the two groups with respect to terminal elimination half-life of 6MP.

Liver enzymes, AST and ALT, were significantly higher in the symptomatic patients (AST, $P = 0.001$ and ALT, $P = 0.01$; Table I). Other liver function tests such a GGT, alkaline phosphatase, and total and direct bilirubin were normal in the asymptomtic patients. Jaundice was not reported in the asymptomatic patients.

No statistically significant difference was found in the 6TGN values and in the methylated metabolites of 6MP in the red blood cells between the 4 symptomatic and 4 asymptomatic (not measured in 1 of the 5) patients. TPMT activity measured in the 4 symptomatic patients (25.1–33.7 units/ml pRBC) were within the range indicative of homozygous high TPMT activity, i.e.; >10 units/ml pRBC.

TABLE I. Patients' Characteristics, Laboratory Values, and Pharmacokinetic Parameters

	Patients with GI symptoms					Patients without GI symptoms					
	1	2	3	4	Mean	1	2	3	4	5	Mean
Age (yrs)	5.5	5.5	6.5	11	6.9 ± 2.6	11.5	12	10.5	3	17	10.8 ± 5.0
Sex	F	F	F	M		M	F	M	F	F	
Dose (mg/m^2)	56	52	85	100	73 ± 23	72	40	71	74	66.6	64.7 ± 14.1
6MP peak (μg/L)	39	9.8	60	65	43.5 ± 25.1	322	13.6	34	178	87	127 ± 126
AUC[a]	133.7	219.0	94.4	104.6	137.9 ± 56.6	312	74	105	435	178	221 ± 151
AST (IU/L)	385	174	228	206	248 ± 94	47	47	50	76	17	47 ± 21
ALT (IU/L)	339	558	290	204	348 ± 151	123	102	34	134	32	85 ± 49
Bilirubin μmol/L	243	30	162	61	124 ± 97	N	N	N	N	N	N
Direct bilirubin μmol/L	213	22	141	39	104 ± 90	N	N	N	N	N	N
TMPT activity[b]	26.7	33.7	28.5	25.1	28.5 ± 3.7	ND[e]	ND	ND	ND	ND	ND
meMPN[c]	18,662	21,151	12,202	12,365	16,095 ± 4,517		3,000	27,900	23,900	9,100	15,975 ± 11,841
6TGN[d]	177	171	339	384	268 ± 110		187	246	463	196	273 ± 129

[a]Per mg $6MP/m^2$ (ng × min/ml).
[b]Units/ml pRBC.
[c]pmol × 10^8 RBC.
[d]pmol/8 × 10^8 RBC.
[e]ND, Not done.

TABLE II. Pharmacokinetic Parameters of Patients With No GI Symptoms

Patient no.	Terminal $t_{1/2}$ (mins)	Peak (C_{max}) (ng/ml)	Time to peak (T_{max}) (mins)	AUC per mg 6MP/m^2 (ng × min/ml)
1	[a]	350	60	542
2	—	0	—	0
3	278	290	30	428
4		125	60	335
5		225	60	815
6	[a]	76	30	176
7		128	60	374
8	[a]	200	60	470
9	161	195	60	290
10	49	660	30	520
11	230	202.5	75	524
12	—	255	60	477
13		55	240	172
14	128	255	30	225
15	408	112	60	395
16	[a]	350	60	471
17	224	600	20	608
18	—	190	60	453
19	180	540	60	814
20	—	175	60	75
21	277	322	90	312
22	62	13.6	90	74
23	73	34	180	105
24	173	178	75	435
25	78	87	60	178
Mean	179 ± 105	224.7 ± 172.9	70 ± 48	371 ± 214

[a]Terminal rebound of 6MP levels did not allow calculation of the terminal $t_{1/2}$.

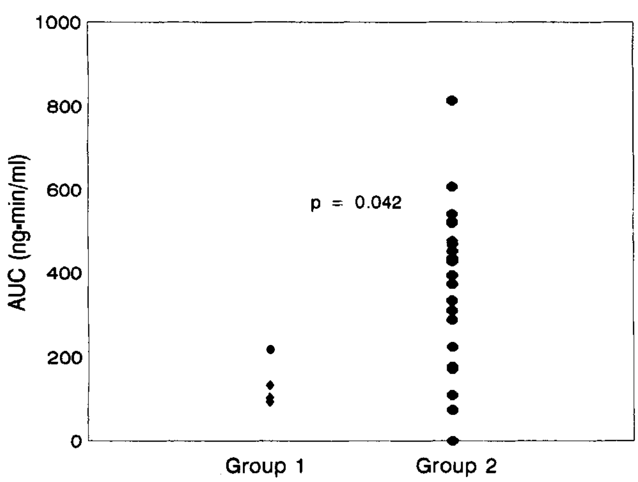

Fig. 1. Group 1 = Patients with gastrointestinal symptoms. Group 2 = Patients without gastrointestinal symptoms. AUC= Area Under the Curve per 1 mg 6MP/m^2.

DISCUSSION

Over 95% of children with ALL achieve remission; however, 30% to 40% will subsequently relapse. Maintenance therapy typically consists of a daily oral dose of 6MP of 75 mg per m^2 of body surface area in addition to treatment with methotrexate, vincristine, and prednisone. 6MP has been reported to be toxic to the bone marrow as well as to the liver and intestine [1–3]. The myelotoxicity of 6MP is related to the intracellular concentration of 6TGN metabolites [11].

The mechanisms leading to 6MP-induced hepatotoxicity are not known at present time. Several other possible causes, unrelated to 6MP therapy, such as viral hepatitis, sepsis, massive infiltration of the liver by leukemic cells, and exposure to other hepatotoxic drugs may play a role in promoting hepatic injury in a leukemic patient. Attempts were made to rule out these etiologies in our patients. Our symptomatic patients received methotrexate, a drug known to be hepatotoxic; however, when 6MP was held, symptoms and signs of hepatitis resolved despite continuation of methotrexate, and a rechallenge with 6MP resulted in similar adverse effects.

The average dose of 6MP was similar in both groups; however, the symptomatic patients had lower 6MP peak concentrations and a longer time to achieve peak plasma levels. Furthermore, the AUC per 1 mg 6MP per m^2 of body surface area was significantly lower in the patients with GI symptoms (Fig. 1). These differences may be explained by impaired absorption of 6MP in the symptomatic patients and/or accumulation of 6MP and its metabolites in their hepatic cells leading to hepatotoxicity.

Of these two mechanisms, impaired absorption should have resulted in lower amounts of 6MP reaching the liver and therefore, lower likelihood of liver damage. No statistically significant difference was found between the 4 patients with hepatotoxicity and the 4 patients without hepatotoxicity in the 6TGN and in the methylated metabolites of 6MP in the red blood cells. This may suggest that absorption of 6MP in the children with hepatotoxicity was not different from those without hepatotoxicity, and was not impaired. Unusual accumulation of the drug in the liver may explain both low systemic exposure and liver damage. Alternatively, it is possible that the underlying mechanism leading to liver and gastrointestinal damage is associated with enhanced xanthine oxidase-induced first pass effect; our methodology did not include measurement of its metabolic products. Measurement of 6MP and its metabolites in the hepatic cells may help to answer this question; however, such a method is currently not available.

6MP is metabolized in the liver by the enzymes TPMT and xanthine oxidase to relatively inactive metabolites and by a multienzymatic process to 6TGN [5]. Patients with low TPMT activity have high concentrations of 6TGN in their erythrocytes and therefore, high incidence of hematologic toxic effects, mainly neutropenia [11]. TPMT activity in red blood cells correlates with the level of enzyme activity in other tissues including the liver [12]. The results of measurement of TPMT activity in our patients indicate that they have enzyme activity 10–27 units/ml pRBC, that is consistent with a homozygous wild type genotype for TPMT. However, children with ALL receiving chemotherapy including 6MP generally have increased activity of TPMT consistent with induction of this enzyme during therapy [13]. The RBC concentration of the methylated 6MP nucleotides (meMPN) in our patients was high, as would be expected in patients with high TPMT activity. However, no significant difference was found between the symptomatic and asymptomatic patients. Although the power of four cases with GiT symptoms compared to four controls is limited, the numerical values were identical for both meMPN and 6TGN. Although the meMPN metabolites are considered to be less cytotoxic compared to 6TGN [14] it is possible that they may be selectively toxic to hepatic cells and may induce hepatotoxicity. The number of patients on whom 6TGN and meMPN levels were measured was small, which may have precluded detection of a difference between the asymptomatic and symptomatic groups. Alternatively, 6MP and/or its metabolites may induce, even in normal concentrations, a hypersensitivity reaction, developing after a period of sensitization of several weeks or months, unaccompanied by the traditional hallmarks of hypersensitivity, as a result of a different immunological mechanism. At present time this theoretical mechanism has not been explored.

In order to prevent therapeutic failure and a higher risk of relapse, early identification of patients who manifest hepatotoxicity would be prudent allowing consideration of alternative chemotherapeutic regimens.

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